LUMINESCENCE-BASED METHODS AND PROBES FOR CYTOCHROME P450 ACTIVITY

IN THE CLAIMS

Please amend the claims as follows.

- 1-131. (Cancelled).
- 132. (Previously Presented) A compound that is a substrate of a cytochrome P450 enzyme and a pro-substrate of a luciferase enzyme, wherein the compound is a structural analog of luciferin, dehydroluciferin or luciferol that includes a substitution at the 6' hydroxy site of luciferin or luciferol or the corresponding 6' site of dehydroluciferin, which substitution includes

 C_{1-20} alkoxy or C_{1-20} alkenyloxy wherein the alkoxy and alkenyloxy are substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl; or

 C_{3-20} alkynyloxy; cycloalkoxy, cycloalkylamino, C_{1-20} alkylamino, di C_{1-20} alkylamino, C_{2-20} alkenylamino, di C_{2-20} alkenylamino, C_{2-20} alkenylamino, C_{3-20} alkynylamino, C_{3-20} alkynylamino, or C_{3-20} alkynylamino, wherein each of the above groups are optionally substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl.

- 133. (Previously Presented) A composition comprising a compound of claim 132.
- 134. (Original) The composition of claim 133, further comprising a pyrophosphatase.
- 135. (Cancelled).
- 136. (Cancelled).
- 137. (Original) A compound selected from the group consisting of

luciferin 6' 2-chloroethyl ether;

luciferin 6' benzyl ether

luciferin 6' 4-picolinyl ether;

RESPONSE UNDER 37 C.F.R. § 1.116 – EXPEDITED PROCEDURE

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luciferin 6' 4-trifluoromethylbenzyl ether;

luciferin 6' phenylethyl ether

luciferin 6' geranyl ether

luciferin 6' prenyl ether

luciferin 6' 2-picolinyl ether; and

luciferin 6' 3-picolinyl ether.

138. (Original) The compound according to claim 137 selected from the group consisting of

luciferin 6' benzyl ether;

luciferin 6' phenylethyl ether;

luciferin 6' geranyl ether; and

luciferin 6' prenyl ether.

139. (Previously Presented) The compound according to claim 137 selected from the group consisting of

luciferin 6' 2-chloroethyl ether;

luciferin 6' 4-picolinyl ether;

luciferin 6' 4-trifluoromethylbenzyl ether;

luciferin 6' 2-picolinyl ether; and

luciferin 6' 3-picolinyl ether.

140-167. (Cancelled)

168. (Previously Presented) The composition according to claim 134 wherein the pyrophosphatase is an inorganic pyrophosphatase.

Page 3 Dkt: 341.044US1 169. (Previously Presented) A compound having the formula:

$$R_1$$
 R_2 R_4 R_5 R_6

wherein

 R_1 represents hydrogen, hydroxy, C_{1-20} alkoxy or C_{1-20} alkenyloxy, wherein the alkoxy and alkenyloxy are substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl; or

 R_1 represents C_{3-20} alkynyloxy; cycloalkoxy, cycloalkylamino, C_{1-20} alkylamino, di C_{1-20} alkylamino, C_{2-20} alkenylamino, di C_{2-20} alkenylamino, C_{2-20} alkenylamino, or C_{3-20} alkynylamino, di C_{3-20} alkynylamino, C_{3-20} alkynylamino, or C_{3-20} alkynylamino, or C_{3-20} alkynylamino, wherein each of the above groups are optionally substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl;

 R_2 and R_3 independently represent C or N;

 R_4 and R_5 independently represent S, O, NR_8 wherein R_8 represents hydrogen or C_{1-20} alkyl, or CR_9R_{10} wherein R_9 and R_{10} independently represent H, C_{1-20} alkyl or fluorine;

 R_6 represents CH_2OH ; COR_{11} wherein R_{11} represents hydrogen, hydroxy, C_{2-20} alkenyl, or $-OM^+$ wherein M^+ is an alkali metal or a pharmaceutically acceptable salt; and

 R_7 represents hydrogen, C_{1-6} alkyl, C_{2-20} alkenyl, halogen or C_{1-6} alkoxy; provided that when R_1 is hydroxy, R_7 is not hydrogen, R_{11} is not hydroxy, R_2 and R_3 are not both carbon, and

 R_4 and R_5 are not both S (luciferin);

when R_1 is hydrogen, R_7 is not hydrogen, R_{11} is not hydroxy, R_2 and R_3 are not both carbon, and R_4 and R_5 are not both S (dehydroluciferin); and

when R_1 is hydroxy, R_7 is not hydrogen, R_6 is not CH_2OH , R_2 and R_3 are not both carbon, and R_4 and R_5 are not both S (luciferol).

170. (Previously Presented) A composition comprising a compound of claim 169.

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171. (Previously Presented) The composition of claim 170, further comprising a pyrophosphatase.

- 172. (Previously Presented) The composition according to claim 171 wherein the pyrophosphatase is an inorganic pyrophosphatase.
- 173. (Previously Presented) The compound according to claim 169 selected from the group consisting of

luciferin 6' 2-chloroethyl ether;

luciferin 6' 4-picolinyl ether;

luciferin 6' 4-trifluoromethylbenzyl ether;

luciferin 6' 2-picolinyl ether; or

luciferin 6' 3-picolinyl ether.

- 174. (Previously Presented) A composition comprising a compound of claim 173.
- 175. (Previously Presented) The composition of claim 174, further comprising a pyrophosphatase.
- 176. (Previously Presented) The composition according to claim 175 wherein the pyrophosphatase is an inorganic pyrophosphatase.
- 177. (Previously Presented) The compound according to claim 169 selected from the group consisting of

luciferin 6' benzyl ether;

luciferin 6' phenylethyl ether;

luciferin 6' geranyl ether; and

luciferin 6' prenyl ether.

178. (Previously Presented) A composition comprising a compound of claim 177.

- 179. (Previously Presented) The composition of claim 178, further comprising a pyrophosphatase.
- 180. (Previously Presented) The composition according to claim 179 wherein the pyrophosphatase is an inorganic pyrophosphatase.
- 181. (Previously Presented) The compound according to claim 169 that has the structure

or a salt thereof.

182. (Previously Presented) The compound according to claim 169 that has the structure

or a salt thereof.

183. (Previously Presented) The compound according to claim 169 that has the structure

$$\begin{array}{c|c} & & & \\ & & \\ & & & \\ & & \\ & & & \\ & & \\ & & & \\ & & \\ & & & \\ &$$

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184. (Previously Presented) The compound according to claim 169 that has the structure

or a salt thereof.

(Previously Presented) The compound according to claim 169 that has the 185. structure

or a salt thereof.

(Previously Presented) The compound according to claim 169 that has the 186. structure

or a salt thereof.

187. (Previously Presented) The compound according to claim 169 that has the structure

$$\begin{array}{c|c} & & & \\ & & \\ & & & \\ & & & \\ & & \\ & & & \\ & & \\ & & \\ & & & \\ & & \\ & & \\ & & & \\$$

188. (Previously Presented) The compound according to claim 169 that has the structure

or a salt thereof.

189. (Previously Presented) The compound according to claim 169 that has the structure

or a salt thereof.

- 190. (Previously Presented) A kit for determining the effect of a substance on cytochrome P450 enzyme activity comprising:
- one or more luminogenic compounds wherein the compound is a cytochrome (a) P450 enzyme substrate and a pro-substrate of luciferase enzyme, wherein the compound is a structural analog of luciferin, dehydroluciferin or luciferol that includes a substitution at the 6' hydroxy site of luciferin or luciferol or the corresponding 6' site of dehydroluciferin, which substitution includes

C₁₋₂₀ alkoxy or C₁₋₂₀ alkenyloxy wherein the alkoxy and alkenyloxy are substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl; or

C₃₋₂₀ alkynyloxy; cycloalkoxy, cycloalkylamino, C₁₋₂₀ alkylamino, diC₁₋₂₀ alkylamino, C₂₋₂₀ alkenylamino, diC₂₋₂₀ alkenylamino, C₂₋₂₀ alkenyl C₁₋₂₀ alkylamino, C₃₋₂₀ alkynylamino, diC₃₋₂₀ alkynylamino, C₃₋₂₀ alkynyl C₁₋₂₀alkylamino, or C₃₋₂₀ alkynyl C₂₋₂₀alkenylamino, wherein each of the above groups are optionally substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl; and

(b) directions for using the kit.

- 191. (Previously Presented) The kit according to claim 190, further comprising one or more bioluminescent enzymes.
- 192. (Previously Presented) The kit according to claim 191 wherein the bioluminescent enzyme is a luciferase.
- 193. (Previously Presented) The kit according to claim 191 wherein the bioluminescent enzyme is a firefly or a Renilla luciferase.
- 194. (Previously Presented) The kit according to claim 190 further comprising ATP and magnesium ions.
- 195. (Previously Presented) The kit according to claim 194 further comprising a detergent.
- 196. (Previously Presented) The kit according to claim 195 wherein the detergent is non-ionic.
- 197. (Previously Presented) The kit according to claim 195 further comprising a pyrophosphatase.
- 198. (Previously Presented) The kit according to claim 197 wherein the pyrophosphatase is an inorganic pyrophosphatase.

199. (Previously Presented) The kit according to claim 198 wherein the compound has the formula:

$$R_1$$
 R_2 R_4 R_5 R_6

wherein

 R_1 represents hydrogen, hydroxy, C_{1-20} alkoxy or C_{1-20} alkenyloxy, wherein the alkoxy and alkenyloxy are substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl; or

 R_1 represents C_{3-20} alkynyloxy; cycloalkoxy, cycloalkylamino, C_{1-20} alkylamino, di C_{1-20} alkylamino, C_{2-20} alkenylamino, C_{2-20} alkenylamino, C_{2-20} alkenylamino, or C_{3-20} alkynylamino, di C_{3-20} alkynylamino, C_{3-20} alkynylamino, or C_{3-20} alkynylamino, or C_{3-20} alkynylamino, wherein each of the above groups are optionally substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl;

R₂ and R₃ independently represent C or N;

R₄ and R₅ independently represent S, O, NR₈ wherein R₈ represents hydrogen or C₁₋₂₀ alkyl, or CR₉R₁₀ wherein R₉ and R₁₀ independently represent H, C₁₋₂₀ alkyl or fluorine;

 R_6 represents CH_2OH ; COR_{11} wherein R_{11} represents hydrogen, hydroxy, C_{2-20} alkenyl, or $-OM^+$ wherein M^+ is an alkali metal or a pharmaceutically acceptable salt; and

R₇ represents hydrogen, C₁₋₆ alkyl, C₂₋₂₀ alkenyl, halogen or C₁₋₆ alkoxy; provided that

when R_1 is hydroxy, R_7 is not hydrogen, R_{11} is not hydroxy, R_2 and R_3 are not both carbon, and R_4 and R_5 are not both S (luciferin);

when R_1 is hydrogen, R_7 is not hydrogen, R_{11} is not hydroxy, R_2 and R_3 are not both carbon, and R_4 and R_5 are not both S (dehydroluciferin); and

when R_1 is hydroxy, R_7 is not hydrogen, R_6 is not CH_2OH , R_2 and R_3 are not both carbon, and R_4 and R_5 are not both S (luciferol).

200. (Previously Presented) The kit according to claim 190, further comprising a reversible luciferase inhibitor.

- 201. (Previously Presented) The kit according to claim 200, wherein the reversible luciferase inhibitor is 2-(4-aminopheny1)-6-methylbenzothiazole (APMBT) or 2-amino-46methylbenzothiazole (AMBT).
- 202. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

or a salt thereof.

203. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

or a salt thereof.

204. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

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205. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

$$F_3C$$

or a salt thereof.

206. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

or a salt thereof.

207. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

or a salt thereof.

208. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

209. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

or a salt thereof.

210. (Previously Presented) The kit according to claim 190 wherein the compound has the structure

- 211. (Previously Presented) A kit for determining the effect of a substance on cytochrome P450 enzyme activity comprising:
- (a) one or more luminogenic compounds, wherein the compound is a cytochrome P450 enzyme substrate and a pro-substrate of luciferase enzyme, and the compound is a selected from

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- (b) one or more bioluminescent enzymes;
- (c) a buffer; and
- (c) directions for using the kit.
- 212. (Previously Presented) The kit according to claim 211 wherein the bioluminescent enzyme is a luciferase.
- 213. (Previously Presented) The kit according to claim 211 wherein the bioluminescent enzyme is a firefly or a Renilla luciferase.

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214. (Previously Presented) The kit according to claim 211 further comprising ATP and magnesium ions.

- 215. (Previously Presented) The kit according to claim 214 further comprising a detergent.
- 216. (Previously Presented) The kit according to claim 215 wherein the detergent is non-ionic.
- 217. (Previously Presented) The kit according to claim 215 further comprising a pyrophosphatase.
- 218. (Previously Presented) The kit according to claim 217 wherein the pyrophosphatase is an inorganic pyrophosphatase.
- 219. (Previously Presented) The kit according to claim 211, further comprising a reversible luciferase inhibitor.
- 220. (Previously Presented) The kit according to claim 219, wherein the reversible luciferase inhibitor is 2-(4-aminophenyl)-6-methylbenzothiazole (APMBT) or 2-amino-46-methylbenzothiazole (AMBT).